In re Application of: GEPSTEIN et al
Serial No.: 10/759,734
Filed: January 20, 2004
Office Action Mailing Date: November 5, 2007

Examiner: Anoop Kumar Sing
Group Art Unit: 1632
Attorney Docket: 27395

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-186 and 196-199 are in this Application. Claims 1-175 and 182-186 have been withdrawn from consideration. Claims 176-181 and 196-199 have been rejected. Claims 178-181 have been canceled herewith. Claims 176 and 199 have been amended herewith.

35 U.S.C. § 112 Rejections

The Examiner has rejected claims 176-181 and 196-199 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner states that these claims are vague and indefinite because they are directed at an in-vitro culture of an isolated human cell being in embryoid bodies.

Claims 178-181 have now been cancelled rendering moot the Examiners rejections with respect to these claims.

Claim 176 has now been amended to recite "An in-vitro culture of isolated human embryoid bodies comprising a plurality of non-cystic embryoid bodies each including human cells ..." thereby correctly defining the culture as that including embryoid bodies which include human cells.

Thus, now amended claim 176 defines human cells which are included within isolated EBs and thus, such cells are isolated from their natural environment.

35 U.S.C. § 102 Rejections

The Examiner has maintained the rejections of claim 176-177 under 35 U.S.C. § 102(b) based on Itskovitz-Eldor et al. (Mol. Med. 2000) and rejected newly added claims 196-199 over this reference.

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The Examiner states that Itskovitz-Eldor et al. a large vacuated EB including cardiac muscle cell layer that is pulsing in synchronous rhythm (Figures 4a-b).

The Examiner has also rejected claims 176-181 and 196-199 under 35 U.S.C. § 102(a) over Itskovitz-Eldor et al. (WO 00/70021) as necessitated by Applicant's amendment to the extent that the claim now embrace cells being in plurality of EBs, a limitation that was not previously recited and considered.

The Examiners rejection are respectfully traversed. Claims 178-181 have now been cancelled thereby rendering moot the Examiners rejection with respect to these claims. Claims 176 and 199 have now been amended.

Applicant disagrees with the Examiner's assessment that the prior art teaches a plurality of EBs each having cells that exhibiting a cardiomyocyte phenotype and as such Applicant does not concede to the Examiner's rejections. Notwithstanding from the above and in the interest of expediting prosecution of this case, Applicant has elected to amend the claims to more clearly define the differences between the present invention and the prior art cited by the Examiner.

As is described in Examples of the instant application the present inventors generated isolated EBs which include cells of myocardial lineage.

Generation of the present EBs is described in Example 1 as follows:

> "The cells were then transferred to plastic Petri dishes (Miniplast, Ein Shemer, Israel), at a cell density of about 5'106 cells in a 58 mm dish, where they were cultured under nonadherent conditions for 7–10 days. During this stage the cells aggregated to form embryoid bodies, which were then plated on 0.1 % gelatin-coated culture dishes and observed microscopically for the appearance of spontaneous contractions." (emphasis added)

As is stated above, the EBs of the present invention were generated by culturing human embryonic stem cells for 7-10 days under non-adherent conditions and then transferring the formed EBs to gelatin-coated plates which essentially 'freeze' the morphological state of the EBs.

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The aggregation of cells into embryoid bodies proceeds through several time dependent stages. Upon aggregation of ES cells, differentiation is initiated and the cells begin to a limited extent to recapitulate embryonic development. The aggregate, while first simply appearing as a ball of cells, takes on an increasingly more complex appearance, becoming a hollow ball (cystic embryoid body) 10-20 days into culturing. The aggregated ball next forms internal structures such as a yolk sac, and cardiomyocytes which beat in a rhythmic pattern to circulate nutrients within the increasingly larger embryoid body. Thus, the morphology of an EB, as well as its cellular state (e.g. presence of cardiomyocytes) is largely dependent on the time period of culturing.

The EBs of the present invention were cultured for 7-10 days to a developmental stage prior to the cystic (also termed vacuated) phenotype, since as is clearly stated in the application:

> "approaches utilizing suspension culture of cystic human embryoid bodies are inefficient, have not demonstrated a satisfactory range of cardiac specific structure and function, have not provided isolated human cardiac cells and tissues, have not demonstrated long term cardiac functionality *in-vitro*, and have not demonstrably provided cells and tissues capable of conferring cardiac function when engrafted *in-vivo*." (emphasis added)

The prior art cited by the Examiner, including Itskovitz-Eldor et al.(Mol. Med. 200 and the Itskovitz-Eldor et al. (WO 00/70021) describe a culture of cystic (or vacuated) human embryoid bodies, since at the time it was thought that cardiomyocyte progenitors only appear following the formation of the cystic phenotype.

In fact, reaching this morphological stage was a goal of the study and patent application of Itskovitz-Eldor et al. since at such stage EB morphology facilitates cell lineage identification and characterization.

The molecular medicine paper from 2000 clearly states in the abstract (under the results header) that "here we report the induction in vitro of cystic embryoid bodies from

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human ES cells". The legend of Figure 2 states "expression of cell-specific genes in human cystic embryoid bodies" while the text repeatedly mentions that the EBs used are 20 day old hEBs (see page 91 column 2, line 5), which as is well known in the art are at the cystic or vacuated stage. Finally, the discussion sections starts with "our findings demonstrate that hES cells readily differentiate to Cystic EBs in a similar manner and time scale to those reported for mES cells.

WO 00/70021, which appears to be based on the work presented in the Molecular Medicine paper (and indeed shares its materials and methods), also clearly states that the EBs were cultured to the cystic or vacuated state. Example 1 of this patent application is in fact entitled "Formation of Cystic EBs" and throughout the application, the culturing conditions described are those which would lead to the formation of cystic EBs (e.g. "20 day old hEBs", Example 1). Thus, in the paper and patent application of Itskovitz-Eldor et al. any EBs that include cells exhibiting a cardiomyocyte phenotype are vacuated or cystic EBs.

Further support to that effect is provided by Example 4 of WO 00/70021 which states: "Figs. 4A and B show[s] a large vacuated hEB including cardiac muscle cells ..."

Thus, the teachings of Itskovitz-Eldor et al. do not anticipate the present invention since these teachings teach and stress the importance of culturing the hEBs to the cystic stage, a stage which is avoided by the present inventors and is in fact taught against.

The Examiner has also rejected claims 176-181 and claim 196-199 under 35 U.S.C. § 102(e) as being anticipated by Benvenisty (US Patent 7405353).

The Examiners rejection are respectfully traversed. Claims 178-181 have now been cancelled thereby rendering moot the Examiners rejection with respect to these claims. Claims 176 and 199 have now been amended.

The Examiner states that Benvenisty discloses a method for directed differentiation of human embryonic cells to a specific cell type, including heart cells.

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Form the Examples section of US Patent 7405353, it is clear that Benvenisty did not culture EBs to the stage of cardiomyocyte formation in the EBs. Benvenisty cultured the EBs for 5 days following which the EBs were dissociated and the cells subjected to various factors to induce differentiation of various cell lineages. Benvenisty did not generate EBs having cells of a cardiomyocytic lineage, nor was it an object of this work.

As is clearly stated in the abstract section of this patent, Benvenisty discloses methods for "mapping a pathway of differentiation of a population of embryonic cells" by "exposing the cells to an exogenous factor and measuring gene expression products that are characteristic of a particular cell type or lineage" and that such mapping is effected on "dissociated embryoid bodies which are then exposed to one or more exogenous factors to enrich a culture for a particular cell type".

Benvenisty does not disclose EBs having cells exhibiting a cardiomyocyte function, nor suggest that the EBs formed thereby might include such cells.

It is well known that isolated embryonic stem cells, including those isolated from non-differentiated EBs, can be cultured under conditions suitable for inducing differentiation of such cells into specific lineages.

Various prior art approaches have attempted to use *in-vitro* culturing of embryo-derived as well as embryoid body-derived cells to generate cardiomyocytic cells and tissues.

The present inventors unexpectedly uncovered that the EBs of the present invention, which are cultured for a time period and under conditions suitable for the formation of cells displaying a cardiomyocyte phenotype, could be used to generate cells and tissues displaying far greater cardiac specific differentiation and functionality than any prior art cell cultures.

As is clearly stated in the instant application, cardiomyocytes derived from the novel EBs of the present invention display significant advantages over prior art culture-differentiated cardiomyocytes including a significant increase in proliferative capacity.

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Benvenisty did not attempt to generate EBs having such cells. The cardiac cell markers described by Benvenisty form a part of a large group of markers which can be used for identifying cell lineages resultant from differentiation of cells dissociated from early stage EBs.

In fact, Benvenisty states that "accordingly, in Examples 1 and 2, differentiation of the cells was assayed by expression of 24 cell specific molecular markers that cover all embryonic germ layers and 11 different tissues.

Simply put, Benvenisty did not set out to generate EBs for the purpose of producing cardiomyocytes, nor was it a result of his work, rather, Benvenisty set out to identify conditions that would lead to the formation of specific cell types in cell cultures generated from cells isolated from dissociated EBs.

Thus, US Patent 7405353 does not describe nor suggest EBs having cells displaying a cardiomyocyte phenotype, nor does it describe or suggest methodology which can be used to generate such EB-included cells. As such, US Patent 7405353 does not anticipate now amended claim 176 and its dependents.

35 U.S.C. § 103 Rejections

The Examiner has also rejected claims 176-181 and 196-199 under 35 U.S.C. § 103(a) as being unpatentable over Itskovitz-Eldor et al. (Mol. Med. 2000) in view of Igelmund et al.

The Examiners rejection are respectfully traversed. Claims 178-181 have now been cancelled thereby rendering moot the Examiners rejection with respect to these claims. Claims 176 and 199 have now been amended.

The teachings of Itskovitz-Eldor et al. are discussed above with respect to the 102 rejections.

Igelmund et al. merely teaches that interaction between cardiomyocytes and beating activity can be determined by recording population action potentials.

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Igelmund et al. are moot with respect to the tissue assayed and as such do not add any relevant information with respect to culturing of embryoid bodies.

As such, Applicant is of the opinion that Itskovitz-Eldor et al. (Mol. Med. 2000) and Igelmund et al. do not render obvious the present invention as now claimed.

In view of the above amendments and remarks it is respectfully submitted that claims 176, 177, 186 and 196-199 are now in condition for allowance. A prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Date: March 5, 2008

Encl.:
Petition for Extension (1 Month)